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**U.S. Army Center for Health Promotion
and Preventive Medicine**
(Provisional)



TOXICOLOGICAL STUDY NO. 87-3012-95
EVIDENCE FOR THE CHEMICAL REDUCTION AND BINDING OF TNT
DURING THE COMPOSTING OF CONTAMINATED SOILS
MARCH 1995 - JANUARY 1996

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U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE (Provisional)

The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) lineage can be traced back over a half century to the Army Industrial Hygiene Laboratory which was established at the beginning of World War II under the direct jurisdiction of The Army Surgeon General. It was originally located at the Johns Hopkins School of Hygiene and Public Health with a staff of three and an annual budget not to exceed three thousand dollars. Its mission was to conduct occupational health surveys of Army-operated industrial plants, arsenals, and depots. These surveys were aimed at identifying and eliminating occupational health hazards within the Department of Defense's (DOD) industrial production base and proved to be extremely beneficial to the Nation's war effort.

Most recently, the organization has been nationally and internationally known as the U.S. Army Environmental Hygiene Agency (AEHA) and is located on the Edgewood area of Aberdeen Proving Ground, Maryland. Its mission has been expanded to support the worldwide preventive medicine programs of the Army, DOD and other Federal agencies through consultations, supportive services, investigations and training.

On 1 August 1994, the organization was officially redesignated the U.S. Army Center for Health Promotion and Preventive Medicine and is affectionately referred to as the CHPPM. As always, our mission focus is centered upon the Army Imperatives so that we are optimizing soldier effectiveness by minimizing health risk. The CHPPM's mission is to provide worldwide scientific expertise and services in the areas of:

- Clinical and field preventive medicine
- Environmental and occupational health
- Health promotion and wellness
- Epidemiology and disease surveillance
- Related laboratory services

The Center's quest has always been one of customer satisfaction, technical excellence and continuous quality improvement. Our vision is to be a world-class center of excellence for enhancing military readiness by integrating health promotion and preventive medicine into America's Army. To achieve that end, CHPPM holds everfast to its core values which are steeped in our rich heritage:

- Integrity is our foundation
- Excellence is our standard
- Customer satisfaction is our focus
- Our people are our most valuable resource
- Continuous quality improvement is our pathway

Once again, the organization stands on the threshold of even greater challenges and responsibilities. The CHPPM structure has been reengineered to include General Officer leadership in order to support the Army of the future. The professional disciplines represented at the Center have been expanded to include a wide array of medical, scientific, engineering, and administrative support personnel.

As the CHPPM moves into the next century, we are an organization fiercely proud of our history, yet equally excited about the future. The Center is destined to continue its development as a world-class organization with expanded preventive health care services provided to the Army, DOD, other Federal agencies, the Nation, and the world community.

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Evidence for the Chemical Reduction and Binding of TNT
During the Composting of Contaminated Soils

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REPLY TO
ATTENTION OF

EXECUTIVE SUMMARY
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EVIDENCE FOR THE CHEMICAL REDUCTION AND BINDING OF TNT
DURING THE COMPOSTING OF CONTAMINATED SOILS
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1. The 2,4,6 isomer of trinitrotoluene (TNT) and its environmental transformation products are the most important munitions-derived pollutants encountered at military installations. Large amounts of TNT were released during manufacturing and demilitarization of ordnance, and the resulting contamination is both toxic and remarkably persistent in soil environments. Remediation of sites contaminated with explosives is required by current statutes, and cleanup criteria have been set from 10 to 200 mg/Kg for TNT. Composting of contaminated soils has the potential of accomplishing the cleanups at lower cost than incineration, but questions remain about the ability of these procedures to effect a "permanent" solution. While composting is effective in converting TNT to an assortment of other compounds, and in binding these to soil, little if any of the TNT is mineralized. It is known that TNT can be transformed in soil environments, by oxidation of the methyl group and reduction of nitro groups. This report outlines the evidence for the preeminence of reductive metabolism of TNT during composting and the binding of the resultant amino metabolites in the processes that form soil humus.

Key Abbreviations and Words: TNT and Composting.

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1. REFERENCES. A list of references is in Appendix A.
2. AUTHORITY. Contract No. 73710676-87-3012 states that this work is funded by the U.S. Army Corps of Engineers.
3. PURPOSE. This report outlines the evidence for the preeminence of reductive metabolism of TNT during composting and the binding of the resultant amino metabolites in the processes that form soil humus.
4. GENERAL.
 - a. Introduction.

(1) The United States has characterized 30 large and medium sized TNT-contaminated sites at federal facilities. As a sum, these sites contain over one million yd³ of contaminated soil. The total number of smaller sites contaminated with TNT is not yet known, but it is reported that the Army alone has on the order of 2,000 explosive-contaminated sites that may require remediation (1), and more exists at Navy and Air force facilities.

(2) Similar patterns of contamination are found throughout Europe and the former Soviet Union. The largest sites in the United States are currently being remediated by incineration of the contaminated soil, but less costly methods are required.

(3) The U.S. Army Environmental Center (formerly the U.S. Army Toxic and Hazardous Materials Agency) has developed composting methods for treatment of TNT-contaminated soils. It has been demonstrated that modern composting methods can save about two-thirds of the approximately \$700/yd³ cost of incineration at medium sized installations.

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(4) It is estimated that the costs of incineration can rise to over \$2000/yd³ at smaller sites, but the cost of composting will increase only to about \$500/yd³ (Craig, H. USEPA Region 10. personal communication). Utilization of composting technology could produce savings on the order of \$25 million from just two midwestern sites scheduled to begin remediation in 1997 (Emery, D.D. Bioremediation Service, Inc., Portland, Oregon. personal communication 1996).

b. Background.

(1) Composting is currently the only bioremediation method for explosives-contaminated soils that has been implemented at a site. It has been accepted by the state of Oregon and the U.S. Environmental Protection Agency Region X and is currently being used for remediating the explosives-contaminated lagoon sediments at the U.S. Army Umatilla Depot Activity site at Hermiston, Oregon. Despite its official recognition, the effectiveness of composting processes for the remediation of TNT-contaminated soils is still challenged because the fate of the pollutants and their stability as "bound residues" have not been determined with certainty.

(2) In the most successful of the field composting demonstrations, 30 vol percent of contaminated soil was mixed with an amendment mixture consisting of 30 vol percent cow manure, 25.4 vol percent sawdust, 25.4 vol percent alfalfa, 14.3 vol percent chopped potato waste, and 4.9 vol percent chicken manure (2) and was arranged in windrows (long piles about 2 meters in height and 3 meters in width maintained in tents on asphalt or concrete pads). The windrows were tested in aerated and nonaerated versions, both with daily turning for a period of 40 days. These processes resulted in greater than 98 percent decrease in acetonitrile-extractable and leachable explosives. In addition, acetonitrile-extractable bacterial mutagens were reduced by more than 98 percent, and leachable compounds toxic to *Ceriodaphnia dubia* were reduced by approximately 90 percent (3,4). The toxicity of aqueous leachates to *Ceriodaphnia dubia*, and the mutagenicity of acetonitrile-extracts decreased steadily with the duration of composting (4). Although the composted soil product caused a small inhibition in the germination and development of some plant species, it also caused an initial enhancement of earthworm size and population (5,3). The overall results suggested that plant and animal populations could be reestablished in the land-applied product.

(3) While composting is effective in converting TNT to an assortment of other compounds, little if any of the TNT is mineralized (complete oxidation of the carbon skeleton to CO₂) (6,7). TNT is degraded by natural processes in many soil environments, but the rates of decay are normally slow and the meta-substituted aromatic ring structure is usually modified, rather than destroyed, by these reactions (8,9,10,11,12,13,14,15). TNT can be transformed either by oxidation of the methyl group or reduction of nitro groups (8,9,12,14)

(Fig. 1). While there may be concern that the suite of compounds generated in this manner may still cause adverse health effects (16), current evidence from assays of bacterial mutagenicity and studies of toxicity to cultured hepatoma cells suggests that these compounds are less toxic than TNT (17,18,19).

5. DISCUSSION.

a. Cleavage of the Aromatic Ring.

(1) Biological mineralization of TNT is difficult because the nitro substituents of this compound prevent the reactions that are required to accomplish cleavage of an aromatic ring. Biological cleavage of aromatic rings requires emplacement of phenolic substituents oriented ortho or para to each other (20,21). The combined electron withdrawing power of the 3 nitro groups makes enzymatic hydroxylation (electrophilic attack) difficult, and the meta spacing of the nitro groups ensures that any hydroxyls that are added will also be emplaced meta to each other. Thus, the catechol/hydroquinone type intermediate required for ring cleavage can not be generated directly from TNT. This inherent resistance to ring cleavage is probably the reason for the persistence of TNT in natural environments.

(2) In contrast with the results of field studies, TNT can be biologically mineralized under certain laboratory conditions. The reported reaction sequence begins with reduction of two or more of the nitro groups to primary amines. In subsequent steps, the amino groups undergo transamination reactions that yield a ring structure with meta substituted phenolic substituents. In later steps, rearrangements occur that move the phenolic groups to an ortho configuration allowing the ring to be oxidatively cleaved (22).

(3) Although mineralization of TNT has been demonstrated in simple bacterial culture experiments performed in the laboratory, it appears that TNT is not utilized as a nutrient for microorganisms in more complex natural environments. Laboratory (12,23,24,25) and field (7) composting experiments utilizing [^{14}C] TNT, and amended soils have conclusively shown that only a very small percent (less than 1 percent) of the TNT is mineralized, and that the bulk of the TNT is retained in the compost as transformed products that are bound to organic matter. It is probable that the required sequence of reduction, transamination, isomerization, and oxidation, necessary for mineralization, is disfavored if other food sources are available. In addition, Bradley has concluded (15) that periodic drying of soils, and toxic inhibition by high TNT concentrations, can affect microbial mineralization of TNT.

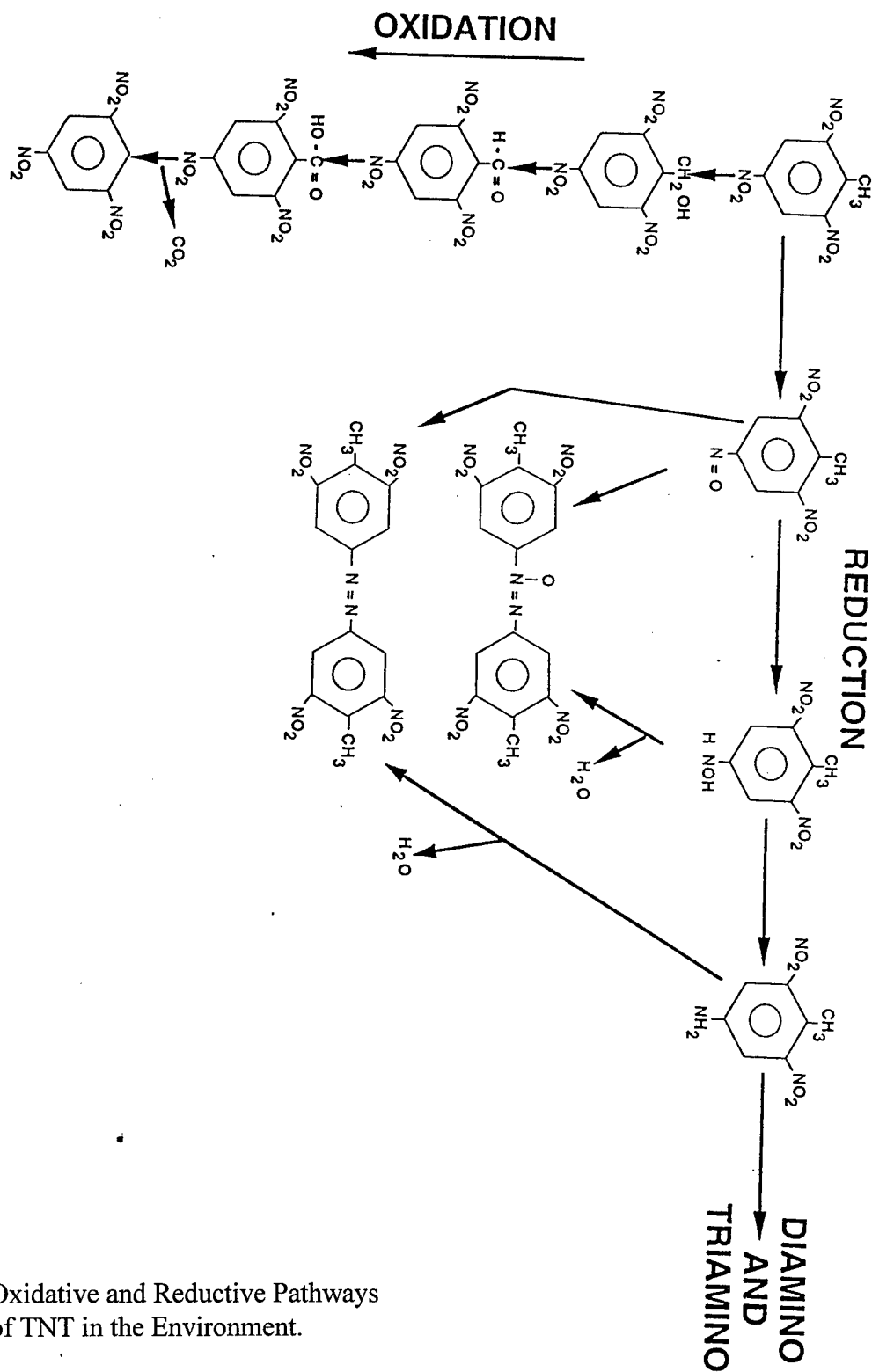


Figure 1. Oxidative and Reductive Pathways of TNT in the Environment.

b. Oxidation of the Methyl Carbon.

(1) TNT contamination in aerobic environments at the surface of the soil is often modified by oxidation of the methyl carbon. This oxidation is probably a multi-step process by which the methyl group is oxidized first to an alcohol, then to an aldehyde, and finally to a carboxylic acid (Fig. 1).

(2) The carboxylic acid function may then leave the ring as CO_2 , yielding trinitrobenzene (TNB). Evidence for the existence of such a pathway was substantiated by Spanggord et al. (26) and Walsh et al. (14). The former reported the formation of trinitrobenzaldehyde and trinitrobenzoic acid during the degradation of TNT to TNB. It has been reported that oxidation of the methyl group of TNT is mediated by surface catalysis on soil minerals (27), by ozonation (28), and by the action of sunlight (26). At sites where the pollution products of TNT reside predominantly at the soil surface, the concentration of TNB may often exceed that of TNT (27).

(3) Although oxidation of the methyl carbon is prevalent in natural environments, it does not appear to be active during the composting process (2,4,19,29). Thus, the TNB that is present in composts of TNT-contaminated soils was probably generated prior to composting. Studies of the fate of TNB during composting have, as yet, not been undertaken.

c. Reduction of the Nitro Group.

(1) Reduction of the nitro groups of TNT has pronounced environmental impact because this process alters the aqueous solubility and, consequently, the rate of leaching of these molecules into groundwater. The monoamine reduction products are less soluble than TNT and the diamino products are more soluble (Amos and Major unpublished). In addition, reduction of the relatively unreactive nitro substituents of TNT produces a suite of substantially more reactive species (14).

(2) Trinitrotoluene is readily reduced to amino-nitro compounds by biological processes. Reduction of the nitro group that resides para to the methyl group (at the #4 position) is more prevalent than reduction of those at the ortho (#2 and #6) positions, as predicted by the work of McCormick et al. (13). It is known that the 4-amino reduction product is the predominant reduced form of TNT in aerobic environments and 2,4-diamino-6-nitrotoluene predominates under mildly acidic (pH 6), anaerobic conditions (16). In addition, it has been reported that the 2,4-diamino reduction product can also be generated under aerobic conditions by reaction with certain minerals associated with clays, and

that this compound is stable in the surface soil environment (30). The triamino reduction product of TNT is not formed at oxygen levels normally encountered in most soil environments (8,16).

(3) At least one nitro group in TNB also appears to be reduced in soil under natural conditions. Walsh et al. (31) reported finding 3,5-dinitroaniline in some TNT-contaminated soils. They hypothesized that the dinitroaniline formed by reduction of TNB derived from the environmental modification of TNT, as described above.

(4) The reduction of the nitro groups of TNT and its monoamine metabolites occurs rapidly in composted soil. In windrow composting monoamine derivatives were observed in the compost as early as the first day of composting (2,4,19) (Fig. 2). In agreement with McCormick (13), the 4-amino reduction product predominated over the 2-amino product. In the second week of composting, diamino products were detected. The 2,4-diamino product appeared at much higher concentration than the 2,6-diamino product (Fig. 2). No other reduction products have been observed to date in acetonitrile extracts of windrow, static pile, and mechanically stirred composts (2,4,19).

(5) Although reports are lacking, it is certain that intermediates in the reduction of nitro substituents occur in composts of TNT-contaminated soils and sediments. The reduction of TNT to amino-nitro compounds is a multi-step process involving nitroso and hydroxylamine intermediates (14) (Fig. 1). Hydroxylamines are chemically unstable compounds that can react with nitroso intermediates to form azoxy bonds. This reaction links the two aromatic rings, forming dimers and perhaps higher level polymers (8,9,13,14,16). Hydroxylamines may also undergo disproportionation reactions in which two hydroxylamines react to yield a nitroso and an amine. The latter compounds may then react abiotically to yield azo compounds (8,9,14).

d. Binding Contaminants to Organic Fractions in Soil or Compost.

(1) The reduction of the hydroxylamine to an amine is important from an environmental standpoint because aromatic amines can react with a variety of functional groups to form covalent bonds that bind these compounds to the organic fraction of soil or compost (Fig. 3). These processes offer the potential for reducing the hazard from TNT-contaminated soils by sequestering transformed products of TNT, as has been suggested for other amino pollutants (32,33).

(2) The reaction pathways for the covalent binding of explosives to composts have not been fully elucidated, but it is likely that reduction of the nitro substituents must occur prior to generation of covalent linkages. Greene et al. (34) found that TNT was retained at higher levels in soil columns that were supplemented with glucose. This experiment supports the

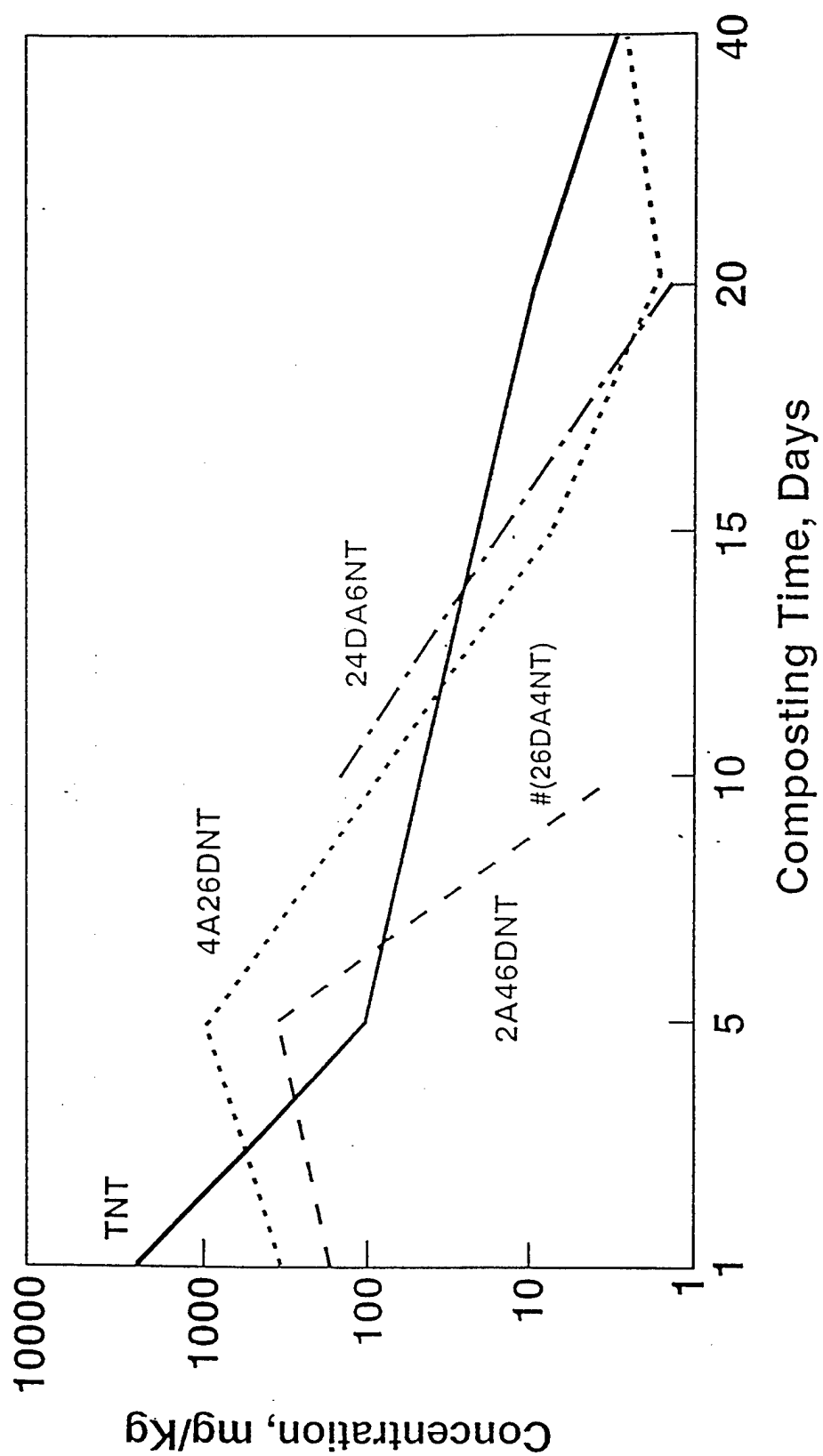


Figure 2. Composting Time.

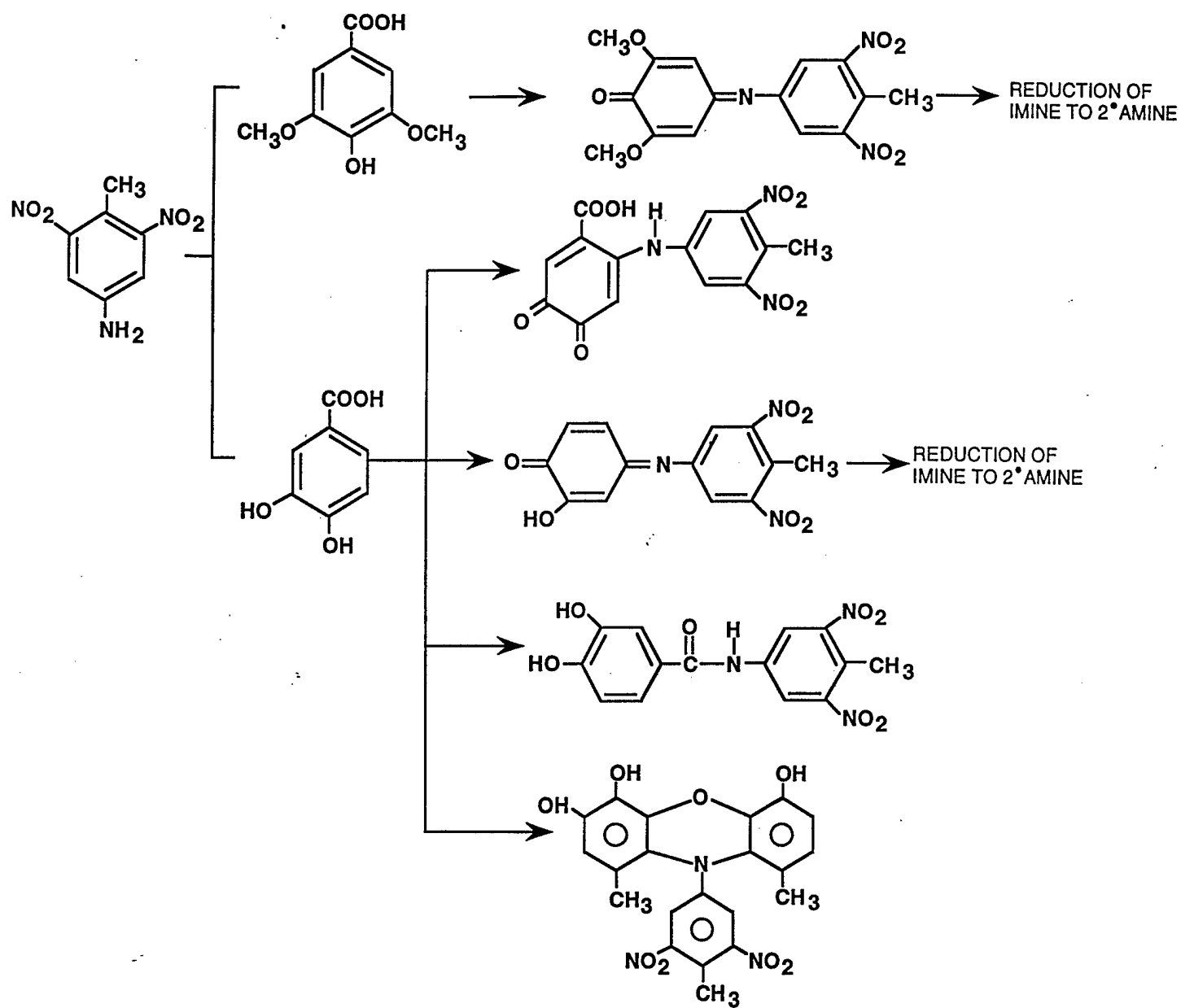


Figure 3. Possible Reactions of Aminonitrotoluenes with Soil Organics.

contention that reduction of nitro groups is needed for binding because addition of glucose to the leaching mixture would tend to promote anaerobic conditions within the column and, thus, increase the rate of reduction. Evidence that reduction of the nitro groups of TNT is necessary for binding during composting is also found in the work of Griest et al. (3,4). These studies compared the decreases in extractable TNT in compost with increases in acetonitrile extractable TNT-reduction metabolites. After 5 days, about 63 percent of the TNT that was lost from the compost was found as extractable amino metabolites. This value dropped to 13 percent by day 10 and 1 percent by day 15, as the metabolites were bound to the matrix.

(3) By analogy with the current understanding of the reactivity and incorporation of aromatic amino-substituted pollutants into soil fractions, the binding process for amino reduction products of TNT could involve at least three mechanisms: the formation of imine bonds by reaction of the amino substituents with carbonyl compounds, the formation of secondary amines, and the incorporation of the amino group into a heterocyclic ring via an intermediate secondary amine (Fig. 3). The importance of these three mechanisms lies in the relative stabilities of their products. The imine reaction is reversible leading to the release of the amino product while the amine and heterocyclic structures are expected to be stable in most soil environments.

(4) The pathway for incorporation of the nitrogen-linked compounds into soil is subject to debate. The work of Bollag suggests that amino compounds are bound to soil by biological processes that generate humus (35,36,37). During this process, natural multi-functional phenols are polymerized in an oxidative process to form soil humus. Reactive intermediates in the oxidation of phenols can also couple with anilines. Thus, aminonitrotoluenes are first bound to phenols and then introduced into the humus complex by the subsequent incorporation of the phenol (Fig. 4). Evidence for such a pathway is found in the work of Pennington et al. (23) who demonstrated that the humus fractions represent the largest class of soil organics to which TNT derivatives are bound during composting.

(5) The rate-limiting step in the humification process is probably the conversion of the phenol to a semiquinone radical (a highly reactive free radical generated with some ortho or para-substituted phenols) (38). Semiquinone radicals react to form alkoxy linkages with other phenols or phenolic acids during the polymerization reactions of the humification process. It has been reported that phenolic free radicals (perhaps semiquinones) also react with aromatic amines to form imine and secondary amine bonds and, thus, serve as a means to link these compounds to the developing humus polymer (35, 39). Evidence that a similar mechanism is involved in linking TNT metabolites to humus has been reported by Major et al. (40). In these studies, both imine and secondary amino linkages were formed between 4-amino-2,6-

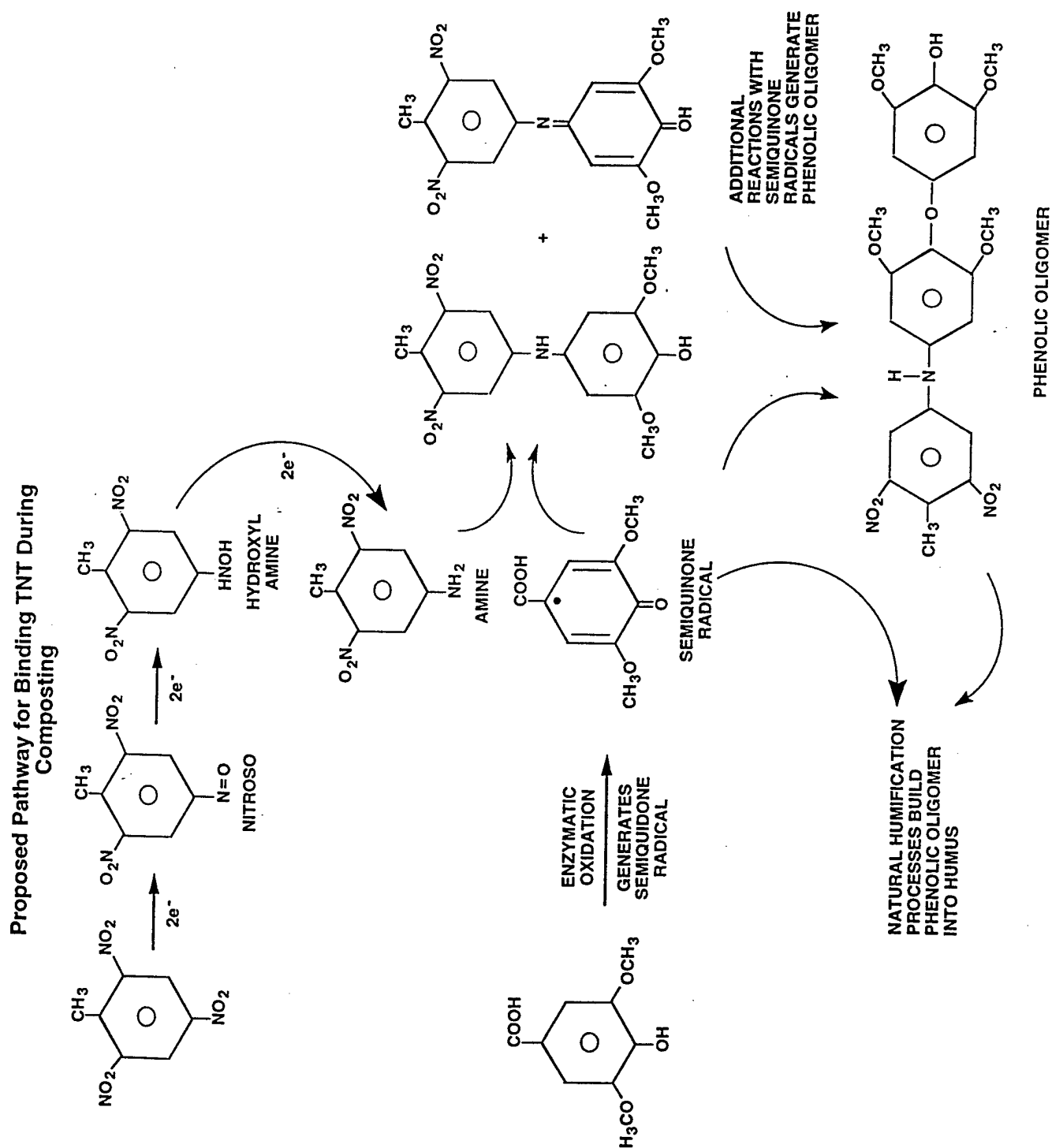


Figure 4. Proposed Pathway for Binding TNT During Composting.

dinitrotoluene and polyfunctional phenols in response to catalysis by horseradish peroxidase. The relative yield of the imine and secondary amine products could be controlled by adjustments in reaction conditions.

(6) Imine bonds would probably fail to provide stable linkages between anilines and soil in many environments, because they are susceptible to hydrolysis (41). A more stable bond may be generated if the imine is subsequently reduced to a secondary amine. The reduction of imine bonds during composting is postulated because reducing conditions are generated periodically during windrow composting.

(7) A second possible route for formation of secondary amine linkages in soil is described by the work of Bartha (42) and Parris (41). In these reports, it is shown that aromatic amines can react by abiotic methods, and that it is not necessary for them to be present when the humus is formed in order to be bound. In the latter work, anilines were reacted with quinones, resulting in the direct formation of a secondary amine linkage between the aniline nitrogen and the ring carbon para to a carbonyl group of the quinone. In addition, Parris (41) and Hsu and Bartha (43) reported mechanisms by which anilines could be linked into stable heterocyclic rings. The proposed reaction mechanisms involve formation of a secondary amine between the aniline nitrogen and a carbon of a substituted aromatic ring. In subsequent reactions, an additional bond forms between other functional groups or between the secondary amine nitrogen and a second reactive center yielding the heterocyclic rings.

(8) Formation of a heterocyclic ring by generation of a tertiary amine is a favored reaction for secondary amines because secondary amines do not form imines (41). Inclusion of one or more of the amino groups that arise from the nitro reduction of TNT into heterocyclic rings, would essentially make the residue an integral part of the soil humus complex.

(9) It is likely that TNT reduction products are bound to humus by both biotic and abiotic mechanisms in natural soil environments and during composting. In environments where the humus forming reactions are slowed (such as in depleted or "finished" composts), the abiotic methods could predominate. However, early phase composting conditions more closely resemble the models of Bollag. Composting, by definition, is a biologically driven process. The rapid breakdown of organic matter provides heat and a constant supply of phenolic monomer to be acted on by the high levels of phenol oxidases present. It is beyond the scope of this report to speculate on the relative contribution of each of the above mechanisms in the binding of TNT residues to soil. It is sufficient for our purposes to outline the current literature and note that all pathways described above utilize the amino metabolites of TNT and yield similar bound products.

e. TNT-Residues in Compost/Soil Systems.

(1) It follows from the above discussion, that the binding of TNT metabolites to soils may depend in large part on generation of amino functional groups by reduction of nitro substituents. In addition, it is known that anaerobic conditions and low pH favor the rapid formation of amino substituents. Large scale composting cycles between oxidative and reductive stages and is, therefore, not ideal for the reduction of nitro groups. Although reduction of nitro groups occurs under aerobic conditions, the reaction is slower than in anaerobic environments and the amount of nitroso and hydroxylamine intermediates probably increases (8,14,16). Therefore, increasing the anaerobic character of composts should accelerate production of amino metabolites and, thus, potentiate the binding process. This theory is supported by the work of Lowe et al. (2) and Griest et al. (3,4), who reported that biotransformation of explosives in windrow composting was more efficient in nonaerated than in aerated windrows.

(2) An increase in the anaerobic character of a compost system would also tend to decrease the concentration of hydroxylamine intermediates. A significant build-up of hydroxylamines would constitute a serious drawback for composting as a remediation method because composts tend to generate the aerobic, alkaline conditions that best support the condensation of hydroxylamines with nitroso intermediates to form azoxy-linked dimers and polymers. Azoxy-linked dimers and higher level polymers are probably bioremediation "dead ends" because they have used their reactive nitrogen substituents in forming the azoxy linkage. These compounds are also poorly soluble in water, and hence, less reactive toward binding processes that occur with phenolic monomer or soluble humates in solution.

(3) Despite the potential for composting to produce azoxy-linked dimers, these compounds were not consistently present in compost systems. Kaplan and Kaplan (12) reported that azoxy compounds can become the principal form of TNT metabolites under simulated composting conditions. However, some studies that used lower concentrations of TNT in the compost showed little accumulation of any TNT reaction products (2,4,19). This is consistent with the work of Isbister et al. (9) who reported that TNT modification products were largely absent even with initial TNT concentrations as high as 10,000 mg/Kg. Other studies showed that concentrations of nitro reduction products often rise early in the composting process and decrease with time (4,29,44) (Fig. 2). In the latter studies, azoxy dimers, though not present in organic solvent extracts, were found in aqueous leachates of "young" (1-5 days of aging) composts (4). In some leachates, the azoxy compounds appeared only after refrigerated storage of the compost leachate samples. These observations suggest that wet conditions, alkalinity, and the presence of oxygen, enhance the formation of azoxy

metabolites. To deter formation and release of azoxy dimers, compost should be protected from rainwater leaching during early active phases, as was practiced in the field demonstration of windrow composting (2).

(4) Current research demonstrates that a variety of bonds may link TNT residues to composts. Although some of these associations are quite strong, some are known to be susceptible to vigorous hydrolytic processes (29,7) and may, therefore, lack sufficient stability to resist degradation by environmental forces. Studies at Oak Ridge of [^{14}C] TNT-inoculated compost showed that 80 percent of the [^{14}C] activity becomes bound to compost particulate by the end of the composting cycle. Caton et al. (7) reported that the majority of the [^{14}C] labeled material is fixed to the soil by bonds that resist extraction with organic solvent but are broken by alkaline hydrolysis. More stable linkages are also formed, but these are generated much more slowly and involve only a small portion (3 to 5 percent) of the TNT.

(5) Recent studies conducted at the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) yielded different results. In these studies, [^{14}C] TNT was added directly to the soil before mixing with the composting materials. This was a more realistic application of the [^{14}C] TNT than in the previous experiment where the radio tracer was applied just after mixing of the soil and amendments. In the USACHPPM work, less than 1 percent of the [^{14}C] from composted [^{14}C] TNT-contaminated soils could be extracted with organic solvents and only 19 percent to 22 percent could be released by refluxing for 2 hour in a solution of 6 N HCL/methanol (45). These differences in behavior may result from the variations in the method of [^{14}C] TNT inoculation as well as from the differences in the chemistry of alkaline and acid hydrolysis. Acid hydrolysis may be more appropriate for evaluating the stability of these complexes because nitroaromatics are susceptible to attack by strong bases. Therefore, base hydrolysis may not be attacking the linkage between the compost and the TNT residue, but instead, may be attacking the substituents of the TNT metabolite directly. Moreover, most humic fractions are soluble in base and these represent the largest class of soil organics to which TNT derivatives are bound during composting. Thus, alkaline treatment may appear to release the bound contamination but in actuality may remove the [^{14}C] metabolites as a soluble oligomer with soil organics.

(6) Because of the extreme complexity of compost, and the resultant difficulty in instrumental analysis of individual polymeric or oligomeric species, the full scope of metabolites of TNT that are produced during composting and the linkages that bind these metabolites to compost have not yet been determined. However, it is important to note that repetitive aqueous leaching of the compost and also ultraviolet light irradiation followed by leaching suggest that TNT residues should not be released by the action of acid rain or sunlight (7).

6. CONCLUSION

a. TNT residues in composted soils undergo transformation, the products of which become bound to organic fractions in the soil or soil amendments. By analogy with the soil binding of other aromatic amines, at least three binding types are expected but have not been demonstrated. It is likely that a humification mechanism is responsible for the binding but other possibilities exist.

b. Toxicological studies on composts of TNT contaminated soils indicate that composting is probably a safe and effective treatment for TNT-contaminated soils. It would be unfortunate if implementation of this technology would be delayed pending the completion of further research. However, further research is called for and could proceed in parallel with the engineering improvements common in a newly developed technology. A more thorough understanding of the chemistry of the composting process can answer concerns over the stability of bound residues. It is important to know the entire suite of metabolites generated in these processes and to identify the covalent binding involved.

7. RECOMMENDATION. The authors recommend further studies using modern nuclear magnetic resonance (NMR) procedures. An examination of humate fractions formed during the composting of C^{13} and N^{15} labeled TNT could do much to clarify the picture. A better understanding of the reactions involved will not only answer questions concerning the stability of bound residues and enable composting conditions to be optimized to minimize formation of undesirable products and produce binding interactions of maximum stability.

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APPENDIX A

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